

# SUPPLEMENTARY DATA

## Table S1

*Saccharomyces cerevisiae* strains used in this study, related to Figures 1-7

## Table S2

Plasmids used in this study, related to Figures 1-7

## Table S3

Oligonucleotides used in this study, related to the Methods section

**Figure S1.** Old model for translation termination and Rli1-GFP Co-IP. (A) Current model for translation termination and open questions. Step 1: Translation elongation is followed by termination and it is unknown when termination factors associate. Step 2: The ribosome reaches a stop codon and most models suggest that eRF1 associates with the ribosome together with eRF3-GTP as ternary complex. eRF1 recognizes the stop codon. The time point of Dbp5-entry is currently unknown and whether Nup159 or another unknown cytoplasmic ADP-release factor recycles Dbp5. GTP-hydrolysis of eRF3 leads to conformational changes in eRF1 resulting in its proper positioning in the ribosomal peptidyl-transferase center. Step 3: The dissociated eRF3-GDP enables Rli1 entry and the locking of eRF1 in a position that allows peptidyl-tRNA hydrolysis. Step 4: Upon peptide release, Rli1 uses its ATPase activity to recycle eRF1 and the ribosomal subunits. (B) Rli1 physically interacts with Dbp5. Western blot analysis of a Dbp5-IP analyzing the binding of Rli1. eRF1 served as positive and Hem15 as negative control. Related to Figure 2A.

**Table S1. *Saccharomyces cerevisiae* strains used in this study.**

Strain number	Genotype	Source
HKY36	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 his3<math>\Delta</math>200</i>	[1]
HKY124	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 his3<math>\Delta</math>200 rat7-1</i>	[2]
HKY130	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 trp1<math>\Delta</math>63 rat8-2</i>	[3]
HKY133	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 his3<math>\Delta</math>200 kap123::HIS3</i>	[4]
HKY291	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 his3<math>\Delta</math>200 trp1<math>\Delta</math>63</i>	[5]
HKY314	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0</i>	Euroscarf
HKY400	<i>MAT<math>\alpha</math> ura3-52 trp1-<math>\Delta</math> lys2-20 leu2-3,112 his4-713 met2-1 tef2-<math>\Delta</math>2 tef1::LEU2 + pCEN tef2-9 URA3</i>	[6]
HKY445	<i>MAT<math>\alpha</math> ura3-1 his5-2 can1-100</i>	[7]
HKY446	<i>MAT<math>\alpha</math> ura3-1 ade2-1 his5-2 can1-100 sup45-2</i>	[7]
HKY473	<i>MAT<math>\alpha</math> ura2 leu2 ade2 trp1 lys2 sup45-2</i>	[5]
HKY474	<i>MAT<math>\alpha</math> ura2 leu2 trp1</i>	This study
HKY477	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 his3<math>\Delta</math>200 trp1<math>\Delta</math>63</i>	This study
HKY522	<i>MAT<math>\alpha</math> pheA10 ade2-144 his7-1 lys9-A21 trp1-289 ura3-52 leu2-3 sup35-21</i>	[8]
HKY553	<i>MAT<math>\alpha</math> pheA10 ade2-144 his7-1 trp1-289 ura3-52 leu2-3</i>	[8]
HKY617	<i>MAT<math>\alpha</math> ade2 arg4 leu2-3,112 trp1-289 ura3-52 DBP5:TAP:K.I.URA3</i>	Euroscarf
HKY618	<i>MAT<math>\alpha</math> ade2 arg4 leu2-3,112 trp1-289 ura3-52</i>	Euroscarf
HKY1121	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 NIP1:GFP:HIS3MX6X</i>	Invitrogen
HKY1122	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 PRT1:GFP:HIS3MX6X</i>	Invitrogen
HKY1123	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 RPG1:GFP:HIS3MX6X</i>	Invitrogen
HKY1271	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 RLI1:GFP:HIS3MX6X</i>	Invitrogen
HKY1646	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 trp1<math>\Delta</math>63 his3<math>\Delta</math>200 rat7<math>\Delta</math>N(1-500)</i>	[9]
HKY1907	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 TRP5::KANMX4</i>	Invitrogen
HKY1914	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 PRT1:GFP:HISMX6</i>	This study
HKY1915	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 PRT1:GFP:HISMX6 sup45-2</i>	This study
HKY1921	<i>MAT<math>\alpha</math> ura3-52 leu2<math>\Delta</math>1 his3<math>\Delta</math>200 trp1<math>\Delta</math>63 DBP5::HISMX6 TRP5::KANMX4</i>	This study
HKY1948	<i>MAT<math>\alpha</math> ura3<math>\Delta</math>0 leu2<math>\Delta</math>0 his3<math>\Delta</math>1 met15<math>\Delta</math>0 HCR1-GFP:HISMX6</i>	Invitrogen

**References for Table S1**

1. Winston, F., C. Dollard, and S.L. Ricupero-Hovasse, *Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C*. Yeast, 1995. **11**(1): p. 53-5.
2. Del Priore, V., et al., *A structure/function analysis of Rat7p/Nup159p, an essential nucleoporin of *Saccharomyces cerevisiae**. J Cell Sci, 1997. **110** ( Pt 23): p. 2987-99.
3. Snay-Hodge, C.A., et al., *Dbp5p/Rat8p is a yeast nuclear pore-associated DEAD-box protein essential for RNA export*. EMBO J, 1998. **17**(9): p. 2663-76.
4. Seedorf, M. and P.A. Silver, *Importin/karyopherin protein family members required for mRNA export from the nucleus*. Proc Natl Acad Sci U S A, 1997. **94**(16): p. 8590-5.
5. Gross, T., et al., *The DEAD-box RNA helicase Dbp5 functions in translation termination*. Science, 2007. **315**(5812): p. 646-9.
6. Dinman, J.D. and T.G. Kinzy, *Translational misreading: mutations in translation elongation factor 1alpha differentially affect programmed ribosomal frameshifting and drug sensitivity*. RNA, 1997. **3**(8): p. 870-81.

7. Stansfield, I., et al., *A conditional-lethal translation termination defect in a sup45 mutant of the yeast Saccharomyces cerevisiae*. Eur J Biochem, 1997. **245**(3): p. 557-63.
8. Cosson, B., et al., *Poly(A)-Binding Protein Acts in Translation Termination via Eukaryotic Release Factor 3 Interaction and Does Not Influence [PSI<sup>+</sup>] Propagation*. Molecular and Cellular Biology, 2002. **22**(10): p. 3301-3315.
9. Hodge, C.A., et al., *Rat8p/Dbp5p is a shuttling transport factor that interacts with Rat7p/Nup159p and Gle1p and suppresses the mRNA export defect of xpo1-1 cells*. EMBO J, 1999. **18**(20): p. 5778-88.

**Table S2. Plasmids used in this study.**

Plasmid number	Features	Source
pHK87	<i>CEN LEU2</i>	[1]
pHK88	<i>CEN URA3</i>	[1]
pHK607	<i>CEN lacZ-UAG-luc LEU2</i>	[2]
pHK608	<i>CEN lacZ-luc LEU2</i>	[2]
pHK629	<i>2μ DBP5 URA3</i>	[3]
pHK653	<i>CEN GFP-DBP5 URA3</i>	[3]
pHK666	<i>CEN GFP-DBP5 LEU2</i>	[4]
pHL693	<i>CEN P<sub>Rat8</sub>rat8-2myc LEU2</i>	This Study
pHK698	<i>CEN Rpl25-GFP URA3</i>	[5]
pHK804	<i>CEN P<sub>ADH1</sub>SUP35-GFP URA3</i>	This Study
pHK886	<i>2μ P<sub>RLI1</sub>RLI1-HA TRP</i>	[6]
pHK887	<i>2μ P<sub>RLI1</sub>RLI1-HA LEU2</i>	[6]
pHK1278	pET15b-SUP45Δ1237-1311	This study
pHK1280	pET28a-SUP45	This study
pHK1283	pGEX-4T1-SUP35	This study
pHK1288	pGEX-6P1	GE Healthcare
pHK1289	pGEX-6P1-DBP5	[4]
pHK1292	<i>2μ RLI1-GFP LEU2</i>	This study
pHK1323	<i>CEN P<sub>ADH1</sub>3xMYC-GLE1 URA3</i>	This study
pHK1335	<i>CEN P<sub>ADH1</sub>SUP45-GFP</i>	This study
pHK1397	<i>CEN P<sub>US3</sub>uS3-GFP LEU2</i>	This study
pHK1414	pGEX-6P1-RLI1	This study
pHK1474	<i>CEN RLI1-GFP TRP1</i>	This study
pHK1475	<i>CEN RLI1-GFP LEU2</i>	This study
pHK1617	pGEX 6P1-GST-eRF1	This study
pHK1618	pGEX 6P1-GST-eRF3ΔN165	This study

### References for Table S2

1. Sikorski, R.S. and P. Hieter, *A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae*. Genetics, 1989. **122**(1): p. 19-27.
2. Stahl, G., et al., *Versatile vectors to study recoding: conservation of rules between yeast and mammalian cells*. Nucleic Acids Res, 1995. **23**(9): p. 1557-60.
3. Gross, T., et al., *The DEAD-box RNA helicase Dbp5 functions in translation termination*. Science, 2007. **315**(5812): p. 646-9.

4. Neumann, B., et al., *Nuclear Export of Pre-Ribosomal Subunits Requires Dbp5, but Not as an RNA-Helicase as for mRNA Export*. PLoS One, 2016. **11**(2): p. e0149571.
5. Gadai, O., et al., *Nuclear export of 60s ribosomal subunits depends on Xpo1p and requires a nuclear export sequence-containing factor, Nmd3p, that associates with the large subunit protein Rpl10p*. Mol Cell Biol, 2001. **21**(10): p. 3405-15.
6. Kispal, G., et al., *Biogenesis of cytosolic ribosomes requires the essential iron-sulphur protein Rli1p and mitochondria*. EMBO J, 2005. **24**(3): p. 589-98.

**Table S3. Oligonucleotides used in this study.**

<b>Oligo number</b>	<b>Sequence</b>	<b>Target</b>
HK1109	5'- <b>cgtcgaattc</b> ATGTCGGATTCAAACCAAG -3'	<i>SUP35</i> forward ( <b>EcoRI site</b> )
HK1110	5'- <b>gactcgag</b> TTACTCGGCAATTTTAACAATTTTAC -3'	<i>SUP35</i> reverse ( <b>XhoI site</b> )
HK1144	5'- <b>gtccatATG</b> GATAACGAGGTTGAAAAAATATTG -3'	<i>SUP45</i> forward ( <b>NdeI site</b> )
HK1146	5'- <b>gtctcgag</b> ATGATAACGAGGTTGAAAAAATATTG -3'	<i>SUP45</i> forward ( <b>XhoI site</b> )
HK1147	5'- <b>gtcatcaggatcc</b> TTAAACTTTGTAACGCAGCATG -3'	<i>SUP45Δ25</i> reverse ( <b>BamHI site</b> )
HK1156	5'- <b>gacctcgag</b> TTAAATGAAATCATAGTCGGATCC -3'	<i>SUP45</i> reverse ( <b>XhoI site</b> )
HK1194	5'- <b>ggccctcgagga</b> ATGGCTAGCAAAGGAGAAG -3'	<i>GFP</i> forward ( <b>PstI site</b> )
HK1195	5'- <b>ggccctcgag</b> TTAGCAGCCGGATCC -3'	<i>GFP</i> reverse ( <b>XhoI site</b> )
HK1398	5'- <b>atcgatcc</b> ATGAGATTTGTGTTTCGATGAG -3'	<i>GLE1</i> forward ( <b>BamHI site</b> )
HK1399	5'- <b>ttaggatcc</b> CTAAGGAGACATTTCCGG -3'	<i>GLE1</i> reverse ( <b>BamHI site</b> )
HK1613	5'- <b>tctttatcttcagggcgcc</b> ATGAGATTTGTGTTTCGATGAG -3'	<i>GLE1</i> forward
HK1614	5'- <b>aagctgtcgacggagct</b> CTAAGGAGACATTTCCGGA -3'	<i>GLE1</i> reverse
HK2136	5'- <b>tatagggcgaattggagct</b> CGACTATACGCTG -3'	<i>P<sub>RLI1</sub></i> forward ( <b>SacI site</b> )
HK2137	5'- <b>cgggccccctcgaggctcgac</b> GTACCGGCCGCAAATTAAG -3'	<i>T<sub>CYC1</sub></i> reverse ( <b>SalI site</b> )
HK2920	5'- <b>gcggcaGGGCCcgcggccgctctagaactagtg</b> -3'	uS3 forward ( <b>Apal site</b> )
HK2921	5'- <b>ggtaccGGGCCcccctc</b> -3'	uS3 reverse ( <b>Apal site</b> )

Figure S1

